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A METHOD OF OBTAINING LUMINESCENT SERA

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TECHNICAL TRANSLATION

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A Method of Obtaining Luminescent Sera

by

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A METHOD OF OBTAINING LUMINESCENT SERA

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A method is known of obtaining luminescent sera by means of attaching to them fluorescent isocyanate, dimethylaminonaphthalenesulfochloride and other fluorochromes.

The proposed method consists of the use of dichlortriazinylaminofluorescein as the label, which allows one to replace the difficultly available dye.

Example 1. 1.77g of cyanurchloride is dissolved in 10ml of anhydrous acetone, cooled to 0°C. and with energetic stirring one adds dropwise a solution of 2.91g of aminofluorescein I in 100ml of anhydrous acetone. This is mixed at 0°C for two hours, and having precipitated a light yellow precipitate is filtered and washed with 10ml of anhydrous acetone.

Yield 3.77g (84.3%). m.p. 300°C.

Example 2. 321.3mg of cyanurchloride in 10ml of anhydrous acetone is cooled to 0°C and with careful stirring one adds dropwise a solution of 514.4mg of aminofluorescein in 50ml of anhydrous acetone. This is mixed for two hours at 0°C, and having precipitated a light yellow precipitate of chlohydrate of dichlorotriazinylaminofluorescein is filtered and washed with anhydrous acetone.

Yield 622.3mg (79%). m.p. 300°C.

Example 3. 100ml of native serum is precipitated with ammonium sulfate at 40-50% saturation. After six hours the precipitate is isolated on a Buchner funnel, dissolved in distilled water so that the content of protein was 3-4%, and salt is removed by successive passage of the solution through cation-exchange and anion-exchange resins according to the published method.

Dichlorotriazinylaminofluorescein in a quantity of 15mg per 1g of protein is pulverized an agate mortar with a small amount of 0.1M phosphate buffer (pH 8.6) and is added to a 2-2.5% solution of protein in the same buffer. Conjugation occurs after mixing for six hours at room temperature. Free dye is removed on Sephadex G-25 (course), 0.01M phosphate buffer (pH 7.5). Protein with a content of fluorescein greater than 3.5 moles per mole of protein, causing nonspecific luminescent background, are separated by adsorption on DEAE-cellulose at pH 7.5 (2g DEAE-cellulose with moisture of 65% per 10ml of conjugate). To the serum one adds merthiolate (1:10,000) and sodium chloride to the physiological concentration. After three days the serum is centrifuged (40,000 rpm) and is subjected to lyophilic drying.

Object of the Invention

The method of obtaining luminescent sera by means of labeling of immune antibodies by a fluorochrome, is distinguished by the purpose of replacing the deficient fluorescein-thiocyanate, and using dichlorotriazinylaminofluorescein as the label.

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